Identification and characterization of wheat–wheatgrass translocation lines and localization of barley yellow dwarf virus resistance[1]

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Abstract: Stable introgression of agronomically important traits into crop plants through wide crossing often requires the generation and identification of translocation lines. However, the low efficiency of identifying lines containing translocations is a significant limitation in utilizing valuable alien chromatin-derived traits. Selection of putative wheatgrass–wheat translocation lines based on segregation ratios of progeny from γ-irradiated seed using a standard phenotypic analysis resulted in a low 4% success rate of identifying barley yellow dwarf virus (BYDV) resistant and susceptible translocation lines. However, 58% of the susceptible progeny of this irradiated seed contained a *Thinopyrum intermedium* chromosome-specific repetitive sequence, which indicated that γ-irradiation-induced translocations occurred at high rate. Restriction fragment length polymorphism (RFLP) analysis of susceptible lines containing alien chromatin, their resistant sister lines and other resistant lines showed that more than one third of the progeny of γ-irradiated double monosomic seeds contained wheatgrass–wheat translocations. Genomic in situ hybridization (GISH) analysis of selected lines confirmed that these were wheatgrass–wheat translocation lines. This approach of initially identifying BYDV susceptible deletion lines using an alien chromosome-specific repetitive sequence followed by RFLP analysis of their resistant sister lines efficiently identified resistant translocation lines and localized the BYDV resistance to the distal end of the introgressed *Th. intermedium* chromosome.

Key words: gene introgression, wide crosses, chromosome, repetitive elements, RFLP, *Thinopyrum intermedium*.

Résumé : L'introgression stable de caractères agronomiques d'importance chez des espèces cultivées via des croisements interspécifiques requiert souvent la production et l'identification de lignées portant des translocations. Cependant, la faible efficacité d'identification de lignées ayant des translocations constitue une contrainte importante en vue de l'exploitation de caractères utiles en provenance d'espèces étrangères. La sélection de potentielles lignées porteuses de translocations entre le blé et le *Thinopyrum intermedium* en fonction des ratios de ségrégation chez une progéniture issue de graines ayant subies une irradiation γ et à l'aide d'une analyse phénotypique standard a connu peu de succès. Seules 4 % des lignées de translocations résistantes ou sensibles au virus de la jaunisse nanisante de l'orge (VJNO) étaient ainsi identifiées. Par contre, 58 % des lignées sensibles issues des graines irradiées contenaient des séquences répétitives spécifiques aux chromosomes du *Th. intermedium*, indiquant que des translocations sont fréquemment induites par l'irradiation. Une analyse RFLP sur les lignées sensibles contenant de la chromatine étrangère, sur les lignées soeurs résistantes ainsi que sur d'autres lignées résistantes a montré que plus du tiers de la progéniture de graines doublement monosomiques et irradiées portaient des translocations blé–*Thinopyrum* Au moyen de l'hybridation génomique in situ, les auteurs ont confirmé que des lignées choisies portaient bel et bien des translocations. Cette approche, reposant dans un premier temps sur l'identification de lignées délétées sensibles au VJNO mais porteuses de séquences répétitives spécifiques des chromosomes étrangers suivie de l'analyse RFLP de lignées soeurs résistantes, a permis d'identifier efficacement des lignées de translocation et a permis de localiser la résistance au VJNO sur l'extrémité distale du chromosome du *Th. intermedium*.

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Mots clés : introgression de gènes, croisements interspécifiques, chromosome, éléments répétitifs, RFLP, *Thinopyrum intermedium*.

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Introduction

Introgression of alien chromatin into wheat (*Triticum aestivum*) through wide crossing has been shown to be extremely useful for increasing the genetic diversity in wheat for disease resistance and other traits during the last four decades (Sears 1956; Gustafson and Sears 1993). However, target genes may not be readily transferred to wheat chromosomes due to the lack of recombination between wheat and alien chromosomes (Sharma et al. 1995). Consequently, incorporation of important traits has required the development of alien chromosome translocations. These translocations can be induced by either ionizing radiation, cell culture, *ph* mutants, or by centric breakage and (or) fusion followed by selection of wheat lines containing small alien chromosome segments (Banks et al. 1995; Friebe et al. 1993).

Molecular markers to augment the phenotypic analysis have been used for identification and analysis of wheat–alien translocation lines (Gale et al. 1989; Hohmann et al. 1996; Rogowsky et al. 1991; Schwarzacher et al. 1992). In lines cytologically identified as translocations, molecular cytogenetic tools have been used to determine the presence and absence of alien chromatin and translocation break points between wheat and alien chromosomes (Chen et al. 1998; Hohmann et al. 1996; Wang and Zhang 1996). However, the low efficiency of selecting, through segregation ratios and cytogenetic or molecular analyses, the progeny that retain the desired gene(s) while minimizing linkage drag has been a limitation in utilizing this technology for crop improvement.

Lack of barley yellow dwarf virus (BYDV) resistant wheat germplasm necessitated the introgression of chromatin into soft red winter wheat from the hexaploid wheatgrass *Thinopyrum intermedium* cv. Ohahe (hereafter noted as *Th. intermedium*; genome E^eE^bSt) (Sharma et al. 1989). This introgression led to the development and characterization of the soft red winter wheat substitution line P29 (Sharma et al. 1995). The salient feature of this line is that it contains an introgressed 7E chromosome that confers complete BYDV subgroup II and moderate subgroup I resistance (Anderson et al. 1998). The structural organization of this chromosome has also been determined, and contains a repetitive sequence on the long arm telomere that is absent on all wheat chromosomes (Francki et al. 1997; Anderson et al. 1998).

In this study, we used the wheatgrass chromosome-specific repetitive element (Francki et al. 1997) and restriction fragment length polymorphism (RFLP) markers to identify and characterize wheatgrass–wheat translocation lines. We have demonstrated that molecular genetic analyses of these mutated progeny can efficiently identify and characterize translocation lines containing varying amounts of the alien chromatin. As a result, resistant translocation lines acceptable for incorporation into a wheat-breeding program were identified. This analysis also localized the BYDV resistance to a region of the chromosome segment near the distal end of 7EL.

Materials and methods

Plant lines

The development of P29 the BYDV resistant disomic alien substitution line containing a group 7 alien *Th. intermedium* chromosome has been previously described (Sharma et al. 1995; Sharma et al. 1997). Double monosomic alien substitution line seeds (7D7E) produced by crossing a disomic alien substitution line with wheat were subjected to 12 kR of γ-irradiation (^{60}Co) to induce translocations in the alien chromosome (Sharma et al. 1999). The resulting M1 plants were selfed and the seed collected and planted to generate plants (M2 and subsequent generations) for phenotypic and molecular analyses.

Phenotypic analysis

Plants derived from the irradiated seed and their parental wheat lines, P29 and *Th. intermedium*, were infested with viruliferous *Rhopalosiphum padi* aphids at the 3- to 4-leaf stage. The viruliferous aphids, containing either the subgroup I P-PAV BYDV or the subgroup II NY-RPV BYDV strain, were sprinkled on test plants randomly arranged in trays in growth chambers. These plants were examined after 5–6 h to insure that 5–10 aphids per plant were feeding on all the test plants. Additional aphids were added as needed to ensure that inoculation occurred. The aphids were killed with the insecticide malathion after feeding for 2 days. The plants were allowed to grow for 2 or 3 additional weeks in the greenhouse, after which time the shoot tissue was harvested and the BYDV titer was measured by ELISA (enzyme-linked immunosorbent assay, Clark and Adams 1977; Hammond et al. 1983) using either BYDV-PAV or BYDV-RPV specific antiserum to determine resistance or susceptibility. ELISA values of <0.3 for BYDV-PAV or <0.1 for BYDV-RPV were considered resistant reactions.

Molecular analyses

To detect alien chromatin in progeny derived from the irradiated seed, slot blots were prepared by denaturing 25 ng of genomic DNA isolated (Francki et al. 1997) from resistant and susceptible wheat-alien introgression lines and susceptible wheat lines. This genomic DNA was denatured in 0.4 M NaOH, 10 mM EDTA at 100°C for 10 min, and transferred to Hybond N^+ membranes using a slot-blot apparatus (Schleicher & Schuell, Keene, N.H.). The slot blots were probed with pAW161 (Guidet et al. 1991), a member of the 350–480 bp rye telomeric repetitive sequences (Appels et al. 1986; Xin and Appels 1988) which, in the wheat – *Th. intermedium* introgression lines used in this study, specifically hybridizes to the 7EL telomere (Francki et al. 1997).

Gel blots used for comparative RFLP analysis were prepared by digesting 15–20 μg of genomic DNA isolated from introgression and non-introgression wheat lines, with the restriction endonucleases *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, and *Hin*dIII (Promega, Madison, Wis.). Following electrophoresis, the digested DNA was transferred to Hybond N^+ membranes (Amersham Life Sciences Inc., Arlington Heights, Ill.) as recommended by the manufacturer, and the resulting gel blots probed with 10 DNA markers obtained from either the consensus maps of Gramineae (Van Deynze et al. 1995) or wheat group 7 chromosomes (Gale et al. 1995). These markers were ascribed to the 7E chromosome by detecting the polymorphic band specific to P29 and *Th. intermedium*, but absent in wheat (Francki et al. 1997). The 7E chromosomal segments containing these markers, detected in the putative translocation lines, were arranged according to their relative positions on the wheat group 7 map based on the synteny of these DNA probes among wheat

Table 1. RFLP analysis of putative translocation lines identified by segregation analysis.

		RFLP Markers										
M4 plant	BYDV Response	cdo545	psr119	cdo475	psr152	psr690	psr311	psr129	psr547	rz682	rz508	pAW161
629-3	S	–	–	–	–	–	–	–	–	–	–	–
630-22	R	+	+	+	+	+	+	+	+	+	+	+
633-3	S	+	+	+	–	–	–	–	–	–	–	+
615-23	R	+	+	+	+	+	+	+	+	+	+	+
635-2	S	+	+	+	+	+	+	–	–	–	–	+
620-21	R	+	+	+	+	+	+	ND	+	+	ND	+
601-25	R	+	+	+	+	ND	+	+	+	+	+	+
605-25	R	+	+	+	+	ND	+	+	+	+	+	+
610-1	R	ND	+	ND	+	ND	+	+	+	ND	ND	+
612-4	R	+	+	+	+	ND	+	+	+	+	+	+
613-4	R	+	+	+	+	ND	+	+	+	+	+	+
614-3	R	+	+	+	+	ND	+	+	+	+	+	+
619-4	R	+	+	+	+	ND	+	+	+	+	+	+
622-28	R	+	+	+	+	ND	+	+	+	+	+	+
624-27	R	+	+	+	+	ND	+	+	+	+	+	+
625-21	R	ND	+	ND	+	ND	+	+	+	ND	ND	+
632-21	R	–	–	–	+	ND	+	+	+	+	+	+

Note: Under RFLP Markers, Southern blot hybridization showing the absence (–) or presence (+) of a *Thinopyrum* 7E chromosome-specific DNA fragment. ND, not determined.

group 7 chromosomes (Gale et al. 1995) and across several species of Gramineae (Van Deynze et al. 1995).

Prior to hybridization, the gel blots and slot blots were rinsed briefly in 2× SSC and the DNA was fixed to the membranes by baking at 80°C for 30–60 min. DNA probes, labeled with [^{32}P]dCTP using a random priming labeling kit (Stratagene, LaJolla, Calif.), were hybridized to the gel and slot blots as previously described (Francki et al. 1997).

Genomic fluorescence in situ hybridization (GISH)

The disomic substitution line P29 and several lines determined by the above molecular analyses to be putative translocation lines (632-21 and 169-1, BYDV resistant; 177-1, BYDV susceptible) were characterized by GISH. Root tips from germinating seeds were excised, immersed in ice water for 24 h, and fixed in ethanol : acetic acid (3:1). Mitotic chromosomes were prepared by macerating the fixed root tips with a 2% cellulase (Serva Cat. No. 16419, 1.3 U/mg, Feinbiochemica GmbH & Co.) and 2.5% pectinase (Sigma P4716, 25 U/mg) enzyme solution, and then squashing the root tips in 45% acetic acid. Slides with squashed root-tip cells were treated with RNase in 2× SSC and stabilized in a 4% solution of freshly depolymerized paraformaldehyde in 1× PBS (phosphate-buffered saline).

Genomic DNA from *Pseudoroegneria stipifolia* (PI22295, St genome) was labeled with biotin-16-dUTP using the BioNick Labeling System (Life Technologies, Inc., Gaithersburg, Md.). *Th. intermedium* was not used as a probe because the wheat DNA, used to block hybridization to the wheat chromosomes, cross-hybridizes to the two E genomes and prevents the majority of the E-genome portion of the probe from hybridizing to the alien chromatin. This reduces the hybridization signal to an unusable level. Consequently, the St genome was used as a probe because, unlike the wheatgrass E genomes, it does not cross-hybridize as strongly to the wheat genome (AABBDD), yet hybridizes well to the E and St wheatgrass genomes. Ninety nanograms of labeled DNA was added as probe in a hybridization solution containing 4.5 μg of autoclaved salmon sperm DNA and 10–14 μg autoclaved genomic wheat DNA (*T. aestivum* cv. Chinese Spring, ABD genomes) or a 3:1 wheat – *Th. elongatum* (E genome) mixture as blocking DNA. Hybridization was performed in a thermal cycler (Hybaid Ltd. OmniGene, U.K.) at 37°C in 50% formamide and 2× SSC. Non-specifically bound probe was removed using a stringent wash solution containing 20% formamide and 0.1× SSC at 42°C. The hybridization was detected using fluorescein isothiocyanate (FITC)-avidin D and biotinylated goat anti-avidin D (Vector Labs, A2001 and BA-0300, respectively). The chromosomes were counterstained by 0.02–0.05% propidium iodide in the vectorshield mounting medium.

Results

Characterization of putative translocation lines identified by segregation analysis

M2 families derived from 74 M1 plants of monosomic substitution γ-irradiated seed were evaluated for their BYDV response (Sharma et al. 1999). Twelve M2 families contained all resistant plants, which suggested that these families were putative translocation lines (PTLs) (Sears 1993). To determine if these were translocation lines, 17 M4 plants derived from 8 of these selected M2 families were evaluated for the presence of the alien chromatin using the 7EL telomere-specific repetitive sequence, pAW161. This slot-blot analysis confirmed the presence of alien chromatin in all but one PTL (629-3, Fig. 1).

These PTLs, previously examined with two RFLP markers (Sharma et al. 1999), were subjected to a more thorough RFLP analysis to evaluate the presence and extent of alien chromosome translocations. The gel blots containing PTL genomic DNA were probed with a set of 10 RFLP markers spanning the wheat group 7 chromosome (Francki et al. 1997). In 13 of the 17 PTLs, all of the 7E RFLP markers tested were present indicating that these were a whole substitution line like P29 (Table 1). One resistant (632-21) and two susceptible (633-3 and 635-2) PTLs were identified as translocation lines, since they contained less than the entire alien 7E chromosome (Table 1; Fig. 2). One susceptible line (629-3) did not contain any of the alien chromosomal seg-

ments tested, and the RFLP patterns were identical to wheat, which suggested that the wheatgrass chromosome was missing.

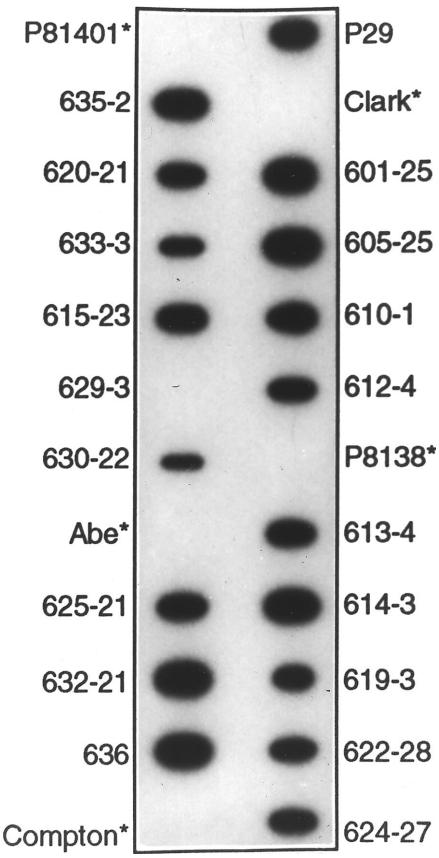
Molecular and cytogenetic characterization of susceptible and resistant deletion lines

Because only three of the 17 PTLs described above were translocations, a second approach was devised for identifying translocation lines. A set of resistant and susceptible M2 lines, which in the previous section had not followed the segregation pattern expected for translocation lines as described by Sears (1993), and consequently were not classified as PTLs, were examined for wheatgrass–wheat translocations. Sixty-six M3 plants, from 40 susceptible and 26 resistant M2 plants, were evaluated for the presence or absence of the 7E long arm telomere-specific repetitive sequence (Table 2). These M2 plants were derived from 28 and 12 M1 plants, respectively. This telomere sequence was present in all 26 plants derived from the resistant M2 plants and was also present in 23 of the 40 plants derived from the susceptible M2 plants. The 17 plants that lacked the 7EL telomere and were susceptible at the M2 generation were not analyzed further, although it is possible that they contained other 7E chromosomal segments.

The presence of alien chromatin in 23 out of a total of 40 susceptible M3 plants clearly demonstrated the BYDV resistance locus on 7E was deleted but wheatgrass DNA was retained. This suggested that a large proportion of the progeny of irradiated seed contained *Th. intermedium* – wheat translocations. These 23 susceptible M3 plants belonged to 16 different M1 families, indicating that at least 69% of the putative translocations were independently derived. From 15 of these 16 M1 families, 19 M4 plants were characterized by RFLP analysis to define these potential translocations. A susceptible line (254-2), which contained alien chromatin but not the 7EL telomere repetitive sequence, was also included in this analysis.

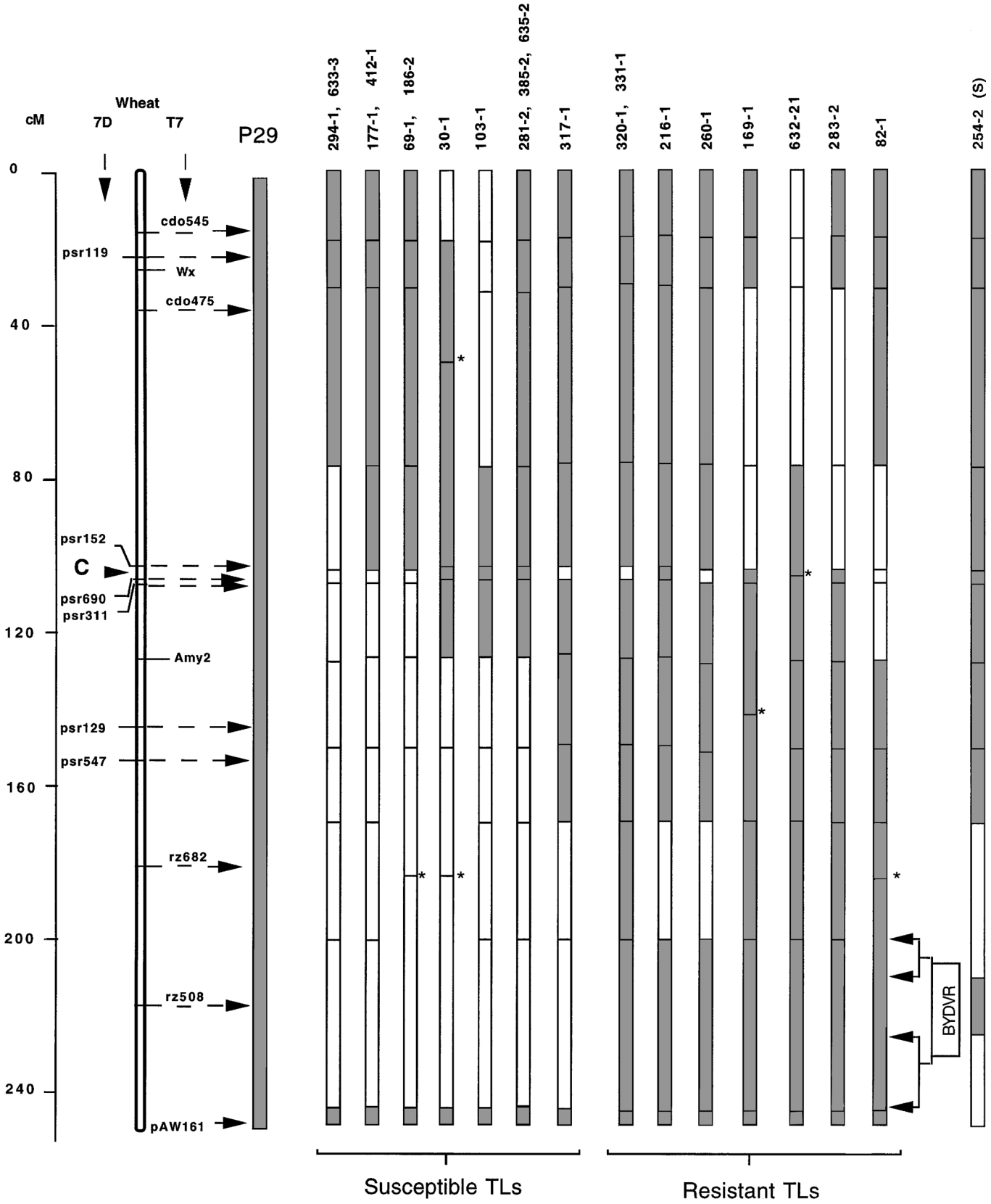
Because we were ultimately interested in identifying BYDV resistant translocation lines, eight resistant sister lines of the susceptible lines containing alien chromatin were included in the RFLP analysis. These lines were examined because we postulated that reciprocal translocations would be present in the resistant sister lines. However, due to the low number of available resistant sister lines, 12 additional resistant M4 plants belonging to 10 additional M1 families, for a total of 18 M1 families, were included in these analyses. In total, therefore, 40 M4 plants (20 resistant and 20 susceptible) were characterized by RFLP analysis. The presence or absence of the RFLP markers (or the chromosomal segments they represent) are shown pictorially on a 7E chromosome according to their relative positions on the wheat group 7 map (Fig. 2). Eleven of the susceptible plants only contained some of the 7E chromosome RFLP markers, identifying these plants as susceptible translocation lines (STLs, Fig. 2). The remaining nine susceptible plants did not contain any of the 7E markers tested, suggesting that these lines were segregating for the alien chromatin or had 7E segments not detectable with the RFLP markers used. The 11 STLs were derived from 10 of the 15 M1 families analyzed indicating that 67% of the 15 M1 families analyzed were STLs. Initially, slot-blot analysis showed that 16 of 28 susceptible M1 families (58%) contained alien chromatin. The combination of the slot-blot and RFLP data determined that at least 38% (0.58 × 0.67 × 100) of the M4 progeny of the γ-irradiated seed contained alien chromosome translocations.

Fig. 1. Slot-blot analysis of putative translocation lines identified by BYDV resistance and susceptibility segregation analysis. This blot was probed with the 7EL telomeric-specific repetitive element, pAW161. Those lines with an asterisk are wheat lines that do not contain *Thinopyrum intermedium* chromatin. Only one line (629-3) did not contain the 7EL telomere, as shown by a lack of hybridization to pAW161.



RFLP analysis revealed that three out of the eight resistant sister lines evaluated (169-1, 216-1, and 283-2) did not contain all the 7E chromosome RFLP markers, confirming that they were resistant translocation lines (RTLs, Fig. 2). The remaining five resistant sister lines contained all the 7E chromosome markers, indicating the lack of 7E chromosome translocations in the regions analyzed by the RFLP markers (data not shown). Similarly, of the 10 additional M1 families analyzed, four M4 plants belonging to three M1 families were confirmed to be RTLs (Fig. 2). In summary, 33% of the resistant lines studied (6 of 18 M1 families) were identified to contain *Th. intermedium* – wheat translocations.

The parental substitution line P29, one susceptible, and two resistant translocation lines were examined by GISH to confirm the RFLP identification of translocations. The parental substitution line P29 showed strong hybridization at the ends of the introgressed *Th. intermedium* chromosome (Fig 3A). The susceptible line 177-1 had 42 wheat chromosomes and one single short chromosome probably consisting



of the short arm of 7E (Fig. 3B), which confirmed the presence of only *Th. intermedium* short-arm molecular markers (Fig.2). In the RTL line 632-21, there was a pair of translocation chromosomes that had a strong hybridization signal on the distal part of the long arm and a weak signal in the middle of the short arm (Fig. 3C). The weak signal observed on the short arm looked much like the rDNA region that could often be seen on chromosome 7D of wheat, indicating

Fig. 2. Schematic diagram showing the presence (grey area) or absence (white area) of 7E chromosomal segments in either BYDV susceptible or resistant translocation lines (TLs). The 7E chromosomal segments in the TLs were arranged assuming the colinearity of Triticeae group 7 markers to the 7E chromosome present in P29; the progenitor of these TLs (Francki et al. 1997). However, it is not known at this point if the wheatgrass translocations are attached to wheat chromosomes other than 7D. The map location of the RFLP markers used in this study as shown with linkage distances (cM) were taken from either the wheat 7D (Gale et al. 1995) or Triticeae group 7 (T7) (Van Deynze et al. 1995) maps. The estimated breakpoints for the segments were placed at half the distance to the neighboring markers. The asterisks indicate that data for that marker for that line was not determined. The location of the BYDV resistance locus (*BYDVR*) was derived from comparing the presence or absence of 7E chromosomal segments between resistant and susceptible TLs and 254-2, a susceptible (S) translocation line that did not contain the wheatgrass-specific telomere repetitive sequence.

that the short arm was 7DS. Due to the lack of a hybridization signal around the centromere of the alien chromosome, the translocation breakpoints could not be determined. RFLP analysis placed the breakpoint on the short arm near the centromere. The GISH of the resistant line 169-1 (Fig. 3D) looked similar to that of the parental substitution line P29, suggesting that this line did not contain a translocation. However, the lack of hybridization signal, or presence of a very weak hybridization signal in the central region of the wheatgrass chromosome neither confirms nor disproves the presence of the RFLP-identified interstitial translocation (Fig. 2). The substitution chromosomes in P29 had a strong hybridization signal at the termini when only wheat DNA was used as the block (Fig. 3A). However, when wheat + *Th. elongatum* (ABD + E) DNA was used as the block, a signal was not observed in P29, 632-21, 169-1, and 177-1 (data not shown). This demonstrates that the hybridization observed in Fig. 3 is specific to the alien chromatin contained in these translocation lines.

Localization of BYDV resistance genes on chromosome 7E

The 7E chromosomal segments of all RDLs and SDLs were arranged according to their relative positions on the wheat group 7 map (Fig. 2). Comparison of the seven resistant and 12 susceptible translocation lines containing varying and overlapping amounts of alien chromatin identified a small chromosomal segment conferring BYDV resistance near the distal end of chromosome 7E. The 7E chromosomal segment containing the wheat group 7L marker, rz508, was present in all the RDLs but was absent in all the SDLs containing the 7EL telomere-specific repetitive sequence (Fig. 2). These results provided strong evidence that the resistance gene(s) were located in the vicinity of this marker on the distal end of chromosome 7EL. The independently identified SDL, 254-2, which did not contain the 7EL telomere-specific repetitive sequence (data not shown), contained the rz508 marker but not its neighboring marker rz682 (Fig. 2). Ordering of the 7E chromosomal segments in all RDLs and SDLs according to their relative positions in wheat group 7 chromosomes localized the BYDV resistance genes to the interstitial region between rz682 and the telomere (Fig. 2).

Discussion

Gamma-irradiation is known to cause major chromosomal changes such as inversions, deletions, and translocations, many of which can be detrimental and lethal (Gustafson and Sears 1993). Identifying deletions or translocations in viable progeny of irradiated material is inefficient, and strategies for developing and analyzing mutated progeny are relatively old (Sears 1956; Stadler 1930). Until recently, segregation analysis of the introgressed trait was the main strategy used for identification of putative translocation lines (Sears 1993; Maluszynski 1990). The difficulty in identifying translocation lines has been a limitation in utilizing the rich genetic diversity in wild relatives for improving crop plants with a narrow genetic base such as wheat. We utilized a wheatgrass-specific repetitive marker and a RFLP strategy to identify, characterize, and determine the frequency of wheat–wheatgrass translocation lines, and localize the BYDV resistance genes on the alien chromosome.

Table 2. Analysis of M3 progeny of γ-irradiated seed for the presence of alien chromatin and response to BYDV infection at the M2 or M4 generation.

Plants tested	7E telomere-specific element	BYDV Response*
26	+	R
0	–	R
23	+	S
17	–	S

Note: R, resistant; S, susceptible. 7E telomere-specific element present (+) or absent (–).

*The M2 parents of these 66 M3 plants were tested for BYDV response, and those M3 plants which contained the 7E telomere were selfed and their M4 progeny tested to confirm their response to BYDV.

A total of 1012 M1 seeds containing the monosomic alien chromosome were irradiated and progeny of 74 M1 seeds were analyzed for their BYDV response. Fifteen resistant and 3 susceptible M4 plants derived from 8 M2 families, which had all resistant plants, were selected as possible translocation lines (PTLs, Sharma et al. 1999). Further analysis on these PTLs using a wheatgrass chromosome-specific repetitive sequence (Fig. 1) and RFLP markers (Table 1) revealed that only one resistant PTL and two susceptible PTLs were confirmed to have deletions. This is a 1.3% success rate for identifying resistant translocation lines. These results were very similar to those obtained by Banks et al. (1995), in which they identified cell culture - induced translocations conferring BYDV resistance at a frequency of 1%.

Molecular and cytogenetic tools have previously been used to characterize wheat–wheatgrass translocation lines containing alien chromatin for identification of specific translocated chromosomal segments (Chen et al. 1998; Friebe et al. 1993; Hohmann et al. 1996; Jiang et al. 1993; Schwarzacher et al. 1992; Wang and Zhang 1996). Hohmann et al. (1996), using molecular cytogenetic tools, characterized nine BYDV resistant translocation lines previously identified by Banks et al. (1995). They were able to group these nine lines into four classes based upon the size of the

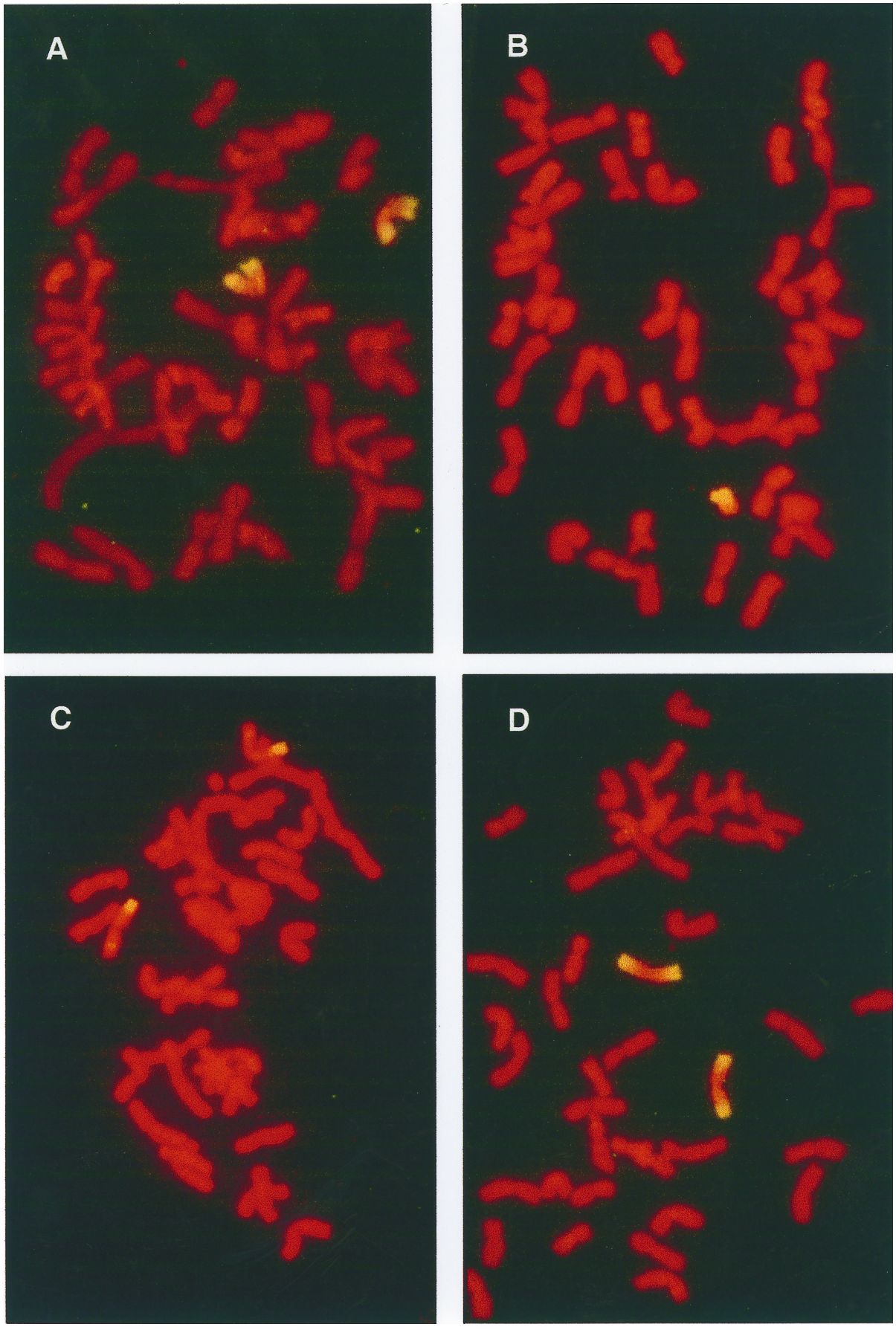


Fig. 3. GISH of mitotic metaphase chromosome spreads of (A) P29, the parental BYDV resistant substitution line, (B) 177-1, a BYDV susceptible deletion line, (C) 632-21, a resistant translocation line, and (D) 169-1, a resistant translocation line. The yellow and orange areas show hybridization of labeled *Pseudoroegneria stipifolia* genomic DNA to the alien chromatin. The hybridization shown in (B) (177-1) identified a short chromosome probably containing just the 7E short arm, hybridization in (C) (632-21) identified a translocation line in which a distal portion of the 7E short arm is missing. In panel (D) (169-1) the hybridization, similarly to P29 (A), is primarily localized to the chromosome ends.

translocation. One class consisted of two lines which appeared to contain an entire wheatgrass chromosome, two classes had the entire long arm and a portion of the short arm of the wheatgrass chromosome, whereas the fourth class consisted of one line that had a portion of the long arm of the wheatgrass chromosome. From a total of 1200 regenerated plants screened and characterized for translocations, only the one line in the fourth class contained a translocation that would be useful in a breeding program.

Our studies also showed that RFLP analysis can provide a powerful strategy to evaluate the amount and location of the alien chromatin in the translocation lines (Table 1; Fig. 2). What has not been addressed in the studies cited above is how to efficiently identify a set of translocation lines from mutated or irradiated plant material. A 1% efficiency of identifying translocation lines (Banks et al. 1995) imposes a significant limitation in the introgression of alien chromatin into crop plants such as wheat.

We have analyzed progeny that were not selected by their adherence to an expected resistance/susceptibility segregation ratio, and identified a large number of lines containing 7E DNA (Table 2) by slot-blot analyses. The evaluation of 40 susceptible M3 plants revealed that as many as 58% of their parental M1 families may contain alien chromosome translocations (Table 2). Since only a telomeric repetitive sequence was used to test for the presence of alien chromatin, this was most likely an underestimation of γ-irradiation-induced translocation. This notion was reinforced by the fact that the independently identified STL (254-2) did not contain the 7EL telomere-specific sequence. The RFLP analysis showed that 67% of the susceptible plants that contained the 7EL telomere had translocations containing different regions of the alien chromosome, demonstrating a success rate of 38% in identifying M4 STLs from irradiated M1 seed. This analysis demonstrated that a large proportion of the progeny, which did not follow the segregation ratio expected for translocation lines (Maluszynski et al. 1990; Sharma et al. 1995; Stadler 1930), were translocation lines. Initially identifying susceptible translocation lines and then analyzing the resistant sister lines also proved to be a useful strategy for identifying resistant translocation lines. Three of the eight resistant sister lines were confirmed to be RTLs, providing a success rate of 42%. RFLP analysis of susceptible lines containing alien chromatin, its resistant sister lines, and other resistant lines showed that more than one third of the progeny of γ-irradiated double monosomic seeds contained wheatgrass–wheat translocations. Therefore, utilization of an alien chromosome-specific repetitive sequence (Table 2) together with a phenotypic analysis of individual plants enhanced the efficiency of identifying translocation lines.

GISH also demonstrated that translocations were present in both resistant and susceptible lines (Fig. 3). This confirmed the results of the molecular marker analysis, which indicated these lines were translocation lines. However, the E and St genomes from *Th. intermedium* and the St genome of *Pseudoroegneria stipifolia* are highly related to the wheat ABD genomes. Consequently, the unlabeled wheat genomic DNA used as a block to prevent hybridization to wheat chromosomes also blocked hybridization to the central region of the alien chromosome. This reduction in hybridization made it difficult to determine the breakpoints in these translocations using GISH. The molecular marker data proved to be more useful in determining the relative position of translocation breakpoints. The RFLP data also identified an interstitial translocation not detectable by GISH.

Banks et al. (1995) and Hohmann et al. (1996) did utilize repetitive elements and RFLP markers, in characterizing putative BYDV resistant translocation lines derived from a wheat line containing an alien wheatgrass chromosome. However, this characterization was only performed on lines that were resistant to BYDV. Furthermore, the repetitive elements used were not wheatgrass specific because when used as a probe in Southern blot hybridizations they identified both wheat and wheatgrass-specific DNA fragments. Consequently, these repetitive markers could not be used as a rapid screening tool for determining the presence of alien chromatin, unlike the wheatgrass-specific telomere repeat used in this study.

Comparative mapping studies have demonstrated extensive colinearity among different species of the family Gramineae. Van Deynze et al. (1995) showed that the map order of the DNA markers from other grass species such as maize and rice is conserved for 92–94% of the length of Triticeae maps, and that this colinearity is increased among closely related species. Employment of this synteny for characterization of closely related species with limited genetic analysis has been advocated but seldom utilized for practical applications. We have previously used this information to characterize the alien chromosome in P29, a wheat disomic–alien substitution line (Francki et al. 1997). The suggested colinearity between wheat and *Th. intermedium*, based on the synteny of the DNA markers across grass species (Van Deynze et al. 1995) allowed us to order the 7E chromosomal segments in the STLs and RTLs relatively to their respective order in wheat (Fig. 2). The position of the alien chromosomal segments in the STLs and RTLs indicated that BYDV resistance is located on short chromosomal segments on the distal end of the 7EL chromosome.

We developed a strategy for analyzing the progeny of γ-irradiated wheat – *Th. intermedium* introgression lines to determine the extent of induced chromosomal changes, identify and characterize translocation lines containing various segments of alien chromosomes, and finally to localize the alien desirable gene(s) to specific chromosomal segments.

This combination of genetic, molecular, and phenotypic analyses has the potential to significantly increase the efficiency of introgressing alien genes into wheat.

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